"A new Method of determining the Number of Micro-organisms in Air." By THOMAS CARNELLEY, D.Sc., Professor of Chemistry, and THOS. WILSON, University College, Dundee. Communicated by Sir HENRY ROSCOE, F.R.S. Received February 3—Read February 16, 1888.

The subject of bacteriology has of late excited considerable interest, and is at present studied by a great number of investigators, both in this country and on the Continent. Under these circumstances a new and improved method for the bacterioscopic analysis of air will be of nterest.

There are several methods at present in use for this purpose, but it will only be necessary to refer to two of these, in both of which solid media are employed.

1. *Hesse's Method* ('Mittheilungen aus dem Kaiserlichen Gesundheitsamte,' vol. 2, p. 182).—This is the oldest process in which a solid medium is used for the nutrition of the micro-organisms, and is the one which has been most commonly employed. The principle of the process consists in drawing a known volume of air through a long wide tube, the inside of which is coated with Koch's nutrient gelatine-peptone. As the air passes through the tube the micro-organisms settle on the jelly, and in the course of a few days develop into zooglea or colonies, and thus become visible to the naked eye and may be counted.

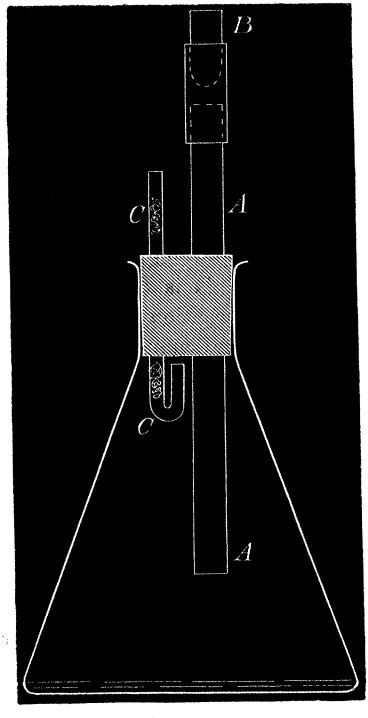
2. *Dr. Percy Frankland's Method* ('Roy. Soc. Proc.,' vol. 41, p. 443; 'Phil. Trans.,' B, vol. 178 (1887), p. 113).—This method consists essentially in aspirating a known volume of air through a small glass tube containing two sterile plugs consisting either of glass-wool alone or of glass-wool coated with sugar. After a given volume of air has been aspirated the two plugs are transferred respectively to two flasks each containing melted sterile gelatine-peptone and plugged with sterile cotton-wool stoppers. The plug is carefully agitated with the jelly so as to avoid any formation of froth, and when the plug has been completely disintegrated and mixed with the gelatine the latter is congealed so as to form an even film over the inner surface of the flask. On incubating these flasks at a temperature of 20° C., the colonies soon begin to appear and may be counted.

*New Method.*—The new process which forms the subject of the present communication is a modification of Hesse's method, in which a flask is substituted for a tube.

The flask employed is conical in form and has a capacity of about half a litre. The flask is fitted with a two-holed india-rubber stopper. Through one hole passes the "entrance tube" AA. This is a piece

of glass tube about 8 inches long and $\frac{3}{8}$ inch* internal diameter. It extends about two-thirds of the way down the flask, and is closed at the outer end by a glass stopper B, fitted on with a piece of india-rubber tubing. Into the other hole of the stopper is fitted the "exit tube" CC. This is simply a piece of ordinary glass tubing (about $\frac{1}{4}$ inch

Fig. 1.




diameter) bent round at the lower end so that it opens in the neck of the flask just under the india-rubber stopper. It is open at both ends, but contains two cotton-wool plugs to prevent any micro-organisms passing back into the flask from the outside air.

10 c.c. of Koch's gelatine-peptone are introduced into the flask and the stopper tied on with copper wire. The flask is then sterilised by heating in steam at 100° C. for an hour and allowed to cool, whereby

* The entrance tube must have at least this width, for if it be too narrow, moisture from the jelly forms during sterilisation on the inside of the tube, and on cooling runs down and collects as a drop on the end, so that the air, on entering the flask, has to pass through this drop of water, which thus retains some of the micro-organisms, and so vitiates the results. This, however, is entirely obviated by using a tube of the prescribed width.

an even layer of gelatine solidifies at the bottom of the flask. On taking the flask out of the steriliser it is generally necessary to carefully rinse the jelly round the sides of the flask so as to take up any steam which may have condensed there and which might subsequently collect in drops and run down on to the colonies and inoculate the rest of the jelly.

In doing this care should be taken to avoid frothing of the jelly.

In taking a sample of air the aspirator is attached to the exit-tube C, and the india-rubber tube and stopper B removed from the end of A. A known volume of air is then drawn through the flask, after which the stopper is replaced. As the air passes through the flask the micro-organisms settle on the jelly, and in the course of a few days develop into colonies and may be counted. If there are a large number of micro-organisms present the bottom of the flask may, for convenience in counting, be marked out into squares with ink. The rate of aspiration we have employed is the same as in Hesse's process, viz., about 1 litre in three minutes. Usually the micro-organisms are deposited more or less directly under the lower end of the entrance tube, while none are deposited on the sides of the flask, even though the latter be coated with jelly, which would seem to indicate that no micro-organisms pass over into the exit tube.

At first sight it seemed very likely that on account of the air having to pass through an entrance tube 8 inches long, a number of the micro-organisms might adhere to the side of the tube and never reach the jelly, so that the results obtained would be too low. In order to ascertain whether this was the case or not, a number of flasks were prepared in which the inside of the entrance tube was coated with a thin layer of jelly. The samples of air were then taken in the usual way, and after sufficient time had been allowed for the development of the colonies, the number in the flask and in the entrance tube were counted, with the following results:—

Table I.

| No. | Circumstances. | Vol. of air taken. | No. of colonies in flask. | No. of colonies in entrance tube. |
|---|---|---|---|---|
| 1 | Dusty air ..... | 400 c.c. | 287 | 3 |
| 2 | Dusty air ..... | 500 „ | 145 | 1 |
| 3 | Dusty air ..... | 500 „ | At least 100 | 4 |

Unfortunately we omitted to count the colonies in No. 3 for a day or two, when it was found that a number of them had run together, but there were at least 100, and probably many more. The above results show that only about 1 per cent. of the micro-organisms

Table II.—Results obtained by Comparative Experiments with Flasks and Hesse Tubes.

| No. | Place. | Date. | Vol. of air taken. | No. of bacteria. | | No. of moulds. | | Total micro-organisms. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | In Hesse tube. | In flask. | In Hesse tube. | In flask. | In Hesse tube. | In flask. |
| | | 1887. | Litres. | | | | | | |
| 1 | Private laboratory | April 21 | 10 | 11 | 11 | 1 | 0 | 12 | 11 |
| 2 | Outside air (Dundee) | ,, 22 | 10 | 2 | 2 | 1 | 1 | 3 | 3 |
| 3 | Outside, beside macerating tubs† | ,, 23 | 10 | 7 | 6 | 6 | 6 | 13 | 12 |
| 4 | Combustion room (dusty air)‡ | ,, 26 | ½ | 54 | 54 | 3 | 4 | 57 | 58 |
| 5 | Long Wynd School, Room 1 | ,, 27 | ½ | 70 | 61 | 1 | 0 | 71 | 61 |
| 6* | Long Wynd School, Room 2 | ,, 27 | ½ | 43 | 22 | 1 | 1 | 44 | 23 |
| 7* | Outside air (Dundee) | ,, 28 | 10 | 45 | 14 | 2 | 0 | 47 | 14 |
| 8 | Vestry of Church§ | ,, 30 | 5 | 11 | 10 | 97 | 86 | 108 | 96 |
| 9* | Outside air (Dundee) | May 2 | 10 | 54 | 26 | 5 | 0 | 59 | 26 |
| 10 | Combustion room (dusty air)‡ | ,, 2 | ½ | 24 | 19 | 0 | 1 | 24 | 20 |
| 11 | Brown Street School, Room 1 | ,, 11 | ½ | 40 | 40 | 1 | 1 | 41 | 41 |
| 12 | Brown Street School, Room 2 | ,, 11 | ½ | 16 | 15 | 0 | 1 | 16 | 16 |
| 13 | Hunter Street School, Room 1 | ,, 12 | ½ | 79 | 95 | 0 | 0 | 79 | 95 |
| 14 | Hunter Street School, Room 2 | ,, 12 | ½ | 94 | 94 | 1 | 0 | 95 | 94 |
| 15* | Balfour Street School | ,, 17 | ½ | 34 | 3 | 3 | 0 | 37 | 3 |
| 16* | Outside, beside macerating tubs† | ,, 19 | 5 | 55 | 11 | 5 | 0 | 60 | 11 |
| 17 | Long Wynd School, Room 1 | ,, 20 | ½ | 19 | 16 | 0 | 0 | 19 | 16 |
| 18 | Long Wynd School, Room 2 | ,, 20 | ½ | 26 | 26 | 2 | 0 | 28 | 26 |
| 19* | Outside air (Dundee) | ,, 21 | 5 | 20 | 5 | 2 | 0 | 22 | 5 |
| 20 | Combustion room (dusty air)‡ | ,, 23 | ½ | 115 | 112 | 16 | 14 | 131 | 126 |
| 21 | Combustion room (dusty air)‡ | ,, 23 | ½ | 71 | 78 | 11 | 10 | 82 | 88 |

* Results non-concordant.

† These experiments were made outside, close to tubs in which a number of animals were macerating for the Biological Museum.

‡ Dusty air produced by shaking door mats.

§ This room is used as a dissecting-room in connexion with the Biological Department. The large number of moulds found in this sample is noteworthy.

adhered to the sides of the entrance tube, even when the latter was coated with jelly, so that under ordinary conditions the number so adhering would probably be very much less. This apparent source of error, therefore, may be entirely neglected when the width of the entrance tube is not less than that prescribed.

In order to test the quantitative accuracy of the method, a number of comparative experiments were made by collecting samples of air simultaneously in the flasks and in Hesse tubes, placed side by side. On p. 458 is a table of the results obtained in this way. In comparing these results it must not be forgotten that, even when two Hesse tubes are compared the one against the other, it is only occasionally that identical numbers are obtained in each tube. Thus one may get six in one tube and eight in the other, or twenty in one tube and twenty-three in the other, and so on, the difference varying according to the total number of micro-organisms present.

From the above table it will be seen that in nearly all cases the number of micro-organisms (both bacteria and moulds) in the tube and in the flask correspond almost exactly. In Nos. 6, 7, 9, 15, 16, and 19, however, this is very far from being the case, for in each of these the flask method gave very much lower results than the Hesse tube. Of these six non-concordant experiments, four were made in outside air, and the other two in schoolrooms in which there was a considerable draught, for the day being warm, the windows and doors were all open.

Now Dr. Percy Frankland (*loc. cit.*) has conclusively proved that Hesse's method does not give reliable results for outside air, except on calm days. He made a number of experiments in which a control tube was used side by side with the aspirated tube, and in this way he was able to obtain a rough idea of the number of micro-organisms which gain access to a Hesse tube, irrespective of aspiration. In illustration of this we may quote a few of his results:—

Table III.

| No. | State of wind | Vol. of air taken. | Micro-organisms in aspirated tube. | Micro-organisms in non-aspirated tube. |
|---|---|---|---|---|
| 1 | Moderate ..... | 12 litres. | 158 | 54 |
| 2 | Slight..... | 12 „ | 12 | 3 |
| 3 | Moderately strong ... | 12 „ | 53 | 11 |
| 4 | Moderately strong ... | 12 „ | 114 | 34 |
| 5 | Moderate, but variable | 12 „ | 49 | 29 |
| 6 | Moderate ..... | 11 „ | 52 | 15 |
| 7 | Strong ..... | 10 „ | 75 | 15 |
| 8 | Strong ..... | 12 „ | 78 | 48 |
| 9 | Slight..... | 12 „ | 72 | 27 |

From these experiments it is evident that Hesse's method is not reliable for outside air, except when there is little or no wind.

By reference to Table II it will be observed that, of the six experiments made in outside air only two were concordant, the discrepancy in the other four being very considerable. In order to learn if this discrepancy was due to the effect of the wind, the state of the latter was ascertained from the Observatory at the Dundee Harbour, for all the dates on which experiments had been made in outside air. The results were as follows :—

Table IV.

| No. | Direction of wind. | Miles per hour. | Wind as felt. | Date. | Micro-organisms in Hesse tube. | Micro-organisms in flask. |
|---|---|---|---|---|---|---|
| 2 | S.W. | 7 | Little or none. | April 22nd. | 3 | 3 |
| 3 | S. | 5½ | Little or none. | April 23rd. | 13 | 12 |
| 7 | S.W. to S. | 6 | Might be gusty. | April 28th. | 47 | 14 |
| 9 | E. to N.E. | 11 | Steady. | May 2nd. | 59 | 26 |
| 16 | W. to S.W. | 13½ | Gusty. | May 19th. | 60 | 11 |
| 19 | W. to N.W. | 9½ | Gusty. | May 21st. | 22 | 5 |

In the two cases in which the number of micro-organisms in the flask corresponded with that in the tube, little or no wind was felt, and the wind was travelling at the rate of about 6 miles per hour; whereas in the other four cases in which discordant results were obtained, the wind was travelling at an average of about 10 miles per hour, and was gusty besides. It would seem, therefore, that the flask method gives more correct results than Hesse tubes for outside air when there is any aerial disturbance.

The only two cases in which there was any discrepancy for inside air were Nos. 6 and 15. Both of these were samples of school air, and it was noted at the time the samples were taken that in both cases there was a considerable draught through the rooms, for the day being warm, the windows and doors were all open. On comparing the determinations of carbonic acid made in these rooms at the same time, it was found that in both they were *comparatively* very low, viz., 10·6 vols. per 10,000 in No. 6, and 7·3 vols. in No. 15; whereas average school air in Dundee contains about 19 vols. of carbonic acid per 10,000. This comparatively low amount of carbonic acid can only be accounted for by the fact that there must have been a draught in the room at the time the experiments were made.

Experiments were also made in order to ascertain if any micro-

organisms gained entrance to the flasks irrespective of aspiration, corresponding experiments being made simultaneously with Hesse tubes. For this purpose a pair of flasks and a pair of Hesse tubes were simultaneously exposed to the outside air for the same length of time, but without aspirating air through any of them. The exit tube (which in an ordinary experiment is connected with the aspirator) of one of each pair of flasks and tubes was stoppered, and the exit tube of the other flask and tube left unstoppered. The entrance to each flask and tube was of course left open. The total number of colonies obtained in each case were as follows, the numbers in brackets being the number of moulds:—

Table V.

| No. | State of wind. | Time of exposure. | Hesse tubes. | | Flasks. | |
|---|---|---|---|---|---|---|
| | | | Stoppered. | Unstoppered. | Stoppered. | Unstoppered. |
| 1 | Very strong..... | ½ hour | .. | 23 [1] | .. | 2 [1] |
| 2 | Gentle ..... | 1 „ | 2 [2] | 1 [1] | 0 | 0 |
| 3 | Gentle .... .. | 1 „ | 6 [5] | 1 [1] | 0 | 0 |
| 4 | Moderately strong and variable.... | ½ „ | 8 [6] | 12 [5] | .. | 0 |
| 5 | Rather strong and variable ..... | ½ „ | 8 [0] | 12 [0] | 0 | 1 [0] |
| 6 | Rather strong and variable ..... | ½ „ | 45 [2] | 33 [1] | 0 | 1 [0] |

Thus out of ten flasks exposed to the air for half to one hour, only three were contaminated, and these only very slightly, and on very windy days, whereas the Hesse tubes were considerably contaminated. It is thus seen that the flask method, unlike the Hesse tube method, is practically free from *vitiation* by aerial disturbance.

We can fully confirm Dr. P. Frankland's statement that Hesse's method gives good results in cases where the air is still and free from draughts, as in most inside buildings and outside on calm still days, for under these conditions Hesse's method agrees remarkably well both with Frankland's process and with our own; whereas in a disturbed atmosphere, as in outside air on windy days, or in buildings where a strong draught prevails, Hesse's method gives results which are considerably in excess of those obtained either by Frankland's method or by our own.

The following are the chief advantages of the new method:—

(1.) It possesses, in common with Hesse's and Frankland's processes, the advantages of a solid nutrient medium.

(2.) It gives accurate results, as shown by comparative tests.

(3.) There is no risk of aerial contamination either during the preparation of the flasks previous to use, or subsequently during the growth of the colonies.

(4.) It is very much cheaper than Hesse's method, for a flask fitted ready for use costs only about 1*s.* 3*d.* (exclusive of jelly), where a Hesse's tube costs about 3*s.* This is a very material item when a large number of experiments are to be made.

(5.) The flasks being of thin glass very rarely break during sterilisation, whereas this is a serious source of annoyance and expense in the case of Hesse's tubes.

(6.) There is not the least chance of leakage during sterilisation, as sometimes occurs with Hesse's tubes, for in the latter method the india-rubber caps have to be very carefully fitted on, since with the slightest crease in the india-rubber the tubes are sure to leak during sterilisation, with consequent loss of jelly, which entails refitting and refilling.

(7.) There is a great saving in jelly. A flask needs only 10 c.c., or one-fifth the quantity required by a Hesse tube. In a long series of experiments the cost of jelly is very considerable, both in the expense of the materials and the time required to make it.

(8.) In common with Frankland's process the flask method is free from errors arising from "aerial currents," which are sometimes so serious a source of error in Hesse's tubes when employed for determinations in outside air, such currents being apt to blow micro-organisms into a Hesse tube over and above those contained in the volume of air aspirated.

(9.) An advantage which the flask method possesses over Frankland's process is that in the former the micro-organisms pass directly on to the nutrient jelly in the flask, whereas in the latter they are first entangled in the glass-wool filter, and afterwards transferred to the cultivating medium, when they are disentangled from the glass-wool by agitation with the jelly, an operation which would seem to require considerable care. Again, in Frankland's process the micro-organisms are embedded in the mass of the jelly, while in our method they fall and grow directly on the surface.

(10.) On the other hand Frankland's method possesses two important advantages; first, on account of the small size of his filter tubes, they admit of being carried from place to place without inconvenience, whereas flasks and Hesse tubes are comparatively bulky. This is a great point when a large number of determinations are to be made at different places away from the laboratory. Second, the air can be aspirated through one of Frankland's filters about four times as fast as through a Hesse's tube, which is of considerable advantage in the case of determinations in outside air, where at least 10 litres require

to be aspirated, though it is of no consequence for the air of buildings where the aspiration of only one-half, or at most 1 litre of air is necessary, and occupies less than two minutes. The rate of aspiration we have employed with our own method has been the same as with Hesse tubes, viz., 1 litre in three minutes. It is not at all unlikely, however, that a more rapid rate might be adopted without affecting the accuracy of the results.

## Addendum. Received April 22, 1888.

The following experiments were made for the purpose of testing whether any micro-organisms pass into the exit tube or become attached to the under side of the cork.

A. *As regards the Passage of Organisms into the Exit Tube.*

In these experiments, the flask was fitted up and charged with jelly in the ordinary manner, except that a little jelly was also placed in the bend of the exit tube. The whole was then sterilised as usual, and, during the subsequent cooling, the flask was so manipulated that a coating of jelly was formed over the inside walls of the exit tube, keeping clear, however, of the cotton-wool plugs. Half a litre of air was then drawn through each flask at the rate of 1 litre in three minutes. The samples were collected in a room in which a slight dust had been raised by the shaking of a door-mat. After the lapse of eight days, the number of colonies counted in each flask was as follows. In no case were any colonies found in the exit tube.

| | Per ½ litre of air. | | |
|---|---|---|---|
| | In flask. | In exit tube. | |
| Experiment I .. | About 300 | 0 | Collected just after raising of dust. |
| Experiment II .. | About 200 | 0 | Collected after a few minutes' interval. |
| Experiment III .. | About 250 | 0 | Collected after a few minutes' interval. |
| Experiment IV .. | About 180 | 0 | Collected after a further interval of a few minutes. |

B. *As regards the Attachment of Organisms to the Under Side of the Cork.*

The flasks were charged and sterilised in the ordinary way, but during cooling, after sterilisation, the flask was so manipulated as to

allow the jelly to form a thin coating over the under side of the cork. Half a litre of air was then drawn through each flask at the rate of 1 litre in three minutes. The samples were collected as before, except that the dust raised was not nearly so great. After nine days, the following number of colonies had developed on the jelly in the flasks, but not a single one was observed on the under side of the cork:—

| | Per ½ litre of air. | | |
|---|---|---|---|
| | In flask. | On cork. | |
| Experiment I .. | 57 | 0 | Collected just after raising of dust. |
| Experiment II .. | 23 | 0 | Collected after an interval of a few minutes. |

The above results show, therefore, that, with an aspiration of 1 litre of air in three minutes, *all* the organisms are deposited on the jelly at the bottom of the flask, and that none reach the cork or exit tube. This result is probably due not only to the action of gravity, but also to the initial velocity, with which the organisms leave the mouth of the entrance tube and enter the flask, being such as to project them on to the surface of the jelly at the bottom of the flask, where they stick and have not the chance of rising again.

FIG. 1.